Abstract

This thesis focuses on two different proteins and their involvement in cellular homeostasis: the transcription factor EB (TFEB), with the effects it has on cellular Ca^{2+} homeostasis, and the c subunit of mitochondrial F_1/F_O ATP synthase, and its important role in the mitochondrial permeability transition pore (mPTP).

TFEB is a master regulator of the lysosomal gene network and it has been demonstrated that the overexpression of TFEB induces lysosomal biogenesis, with a consequent increase of the total lysosomal content per cell, and enhances lysosomal trafficking and their plasma membrane proximity. On the other hand, the recent identification of regulated Ca^{2+} channels in lysosomes suggests that they too may contribute to cytosolic Ca^{2+} signalling. Starting from these observations, we decided to investigate the role of TFEB and the contribution of lysosomes in intracellular Ca^{2+} homeostasis more in depth. We investigated the effects of the transiently TFEB3xflag overexpression on Ca^{2+} homeostasis in HeLa cells, by measuring mitochondrial and cytosolic Ca^{2+} response after agonist stimulation, capacitative calcium entry and Ca^{2+} dynamics in endoplasmic reticulum. No remarkable difference was observed in mitochondrial and cytosolic Ca^{2+} response but we found interesting effects of TFEB overexpression in the capacitative calcium entry. Furthermore, we proved that, if lysosomes are destroyed or damaged by using GPN or Vacuolin-1, these effects are deleted. Moreover, we observed a delay in the calcium uptake time necessary to reach the plateau in endoplasmic reticulum when TFEB is overexpressed. These observations suggest a possible Ca^{2+} buffering role of lysosomes and cast light on a new lysosomal function.

The mitochondrial permeability transition (MPT) is an alteration in the permeability of the mitochondrial inner membrane. Many studies assumed that the permeability transition state is caused by a high permeability channel, namely the mPTP. Although so far the real structure of the mPTP has not been defined clearly, several lines of

evidence suggest that mitochondrial ATP synthase is connected to mPTP. The C subunit of the mitochondrial F1/Fo ATP synthase plays a key role in the activity of the enzyme as it creates the c-ring of the Fo portion. Here, we confirm the theory that considers the c subunit of the mitochondrial F1/Fo ATP synthase as an important player in mPTP formation, with a crucial role in the MPT activity. We used the Blue Native assay to get information about possible conformational arrangements of F1/Fo ATP synthase, which are necessary for the mPTP opening; in particular, we focused on the dimer or monomer state of the enzyme. Then, we considered the possibility of F_1/Fo ATP synthase arrangements in sub-complexes after the induction of mPTP opening and we drew our attention to three F_1/Fo ATP synthase subunits: α, γ and c. Results from the co-IP experiments showed that subunit α, γ and c of ATP synthase interact with each other and that the interaction persists after induction of mPTP opening. Moreover, in order to check if one or more subunits of the enzyme could be fundamental for the mPTP opening, we performed the silencing of several F_1/Fo ATP synthase subunits by the use of siRNA technique in HeLa cells. In association with the silencing, we used Co^{2+}-Calcein assay and fluorescence microscopy to observe mPTP opening after induction with ionomycin. Our results confirmed the involvement of F_1/Fo ATP synthase in the MPT process, in particular in the mPTP structure, and suggest a key role of the c subunit of F_1/Fo ATP synthase in the mPTP opening.